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BACKGROUND

Polycyclic aromatic hydrocarbons (PAHs) are components of crude oil and its by-products and are both recalcitrant as well as carcinogenic (Makker et al, 2003). The chemical, physical and thermal processes are the common techniques involved in the cleaning up of oil contaminated sites (Margesin and Schinner, 2001). These techniques, however, have some adverse effects in the environment and are also expensive (Dendooven et al, 2011). Makker et al (2003) suggested that, of all the clean-up technologies, bioremediation remains the best approach towards remediation of contaminated soils and ground water. Domínguez et al (2000) indicated that bioremediation is environmentally and economic friendly in combating petroleum contaminated sites.

The application of earthworms as agents of bioremediation (vermiremediation) have been reported to be a feasible means of bioremediation due to their involvement in many physical, chemical and biological parameters of the soil based on their activity (Domínguez et al, 2000; Liang et al, 2016). Furthermore, several scholars have reported the ability of earthworms to enhance the removal of several soil contaminants such as polycyclic aromatic hydrocarbons, heavy metals, polychlorinated biphenyl (PCBs) and so on (Contreras-Ramos et al, 2008; Dendooven et al, 2011).



Figure 1: Map of western Nigeria
Source: www.researchgate.com

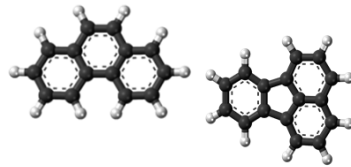


Figure 2: Chemical structures of Phenanthrene and Fluoranthene
Source: www.sanichem.com

AIMS

This research aims to investigate the removal of phenanthrene (PH) and fluoranthene (FL) by earthworms enhanced with rhamnolipid (biosurfactant) and their joint effect on the biochemical processes in epigeic (*Eisenia hortensis*) and anecic (*Lumbricus terrestris*) species of earthworms.

OBJECTIVES

- Assess the biotic and abiotic properties of the soil
- Carry out OECD toxicity test of both PAHs and biosurfactant on earthworm and microtoxicity on indigenous soil microorganisms to determine LC₅₀
- Determine the removal capacity of PAHs by *Eisenia hortensis* and *Lumbricus terrestris* with or without biosurfactant
- Explore and understand the enzymatic metabolism of PAHs, specifically EROD activity of CYP1A1 and their potential as biological indicators.

METHODOLOGY

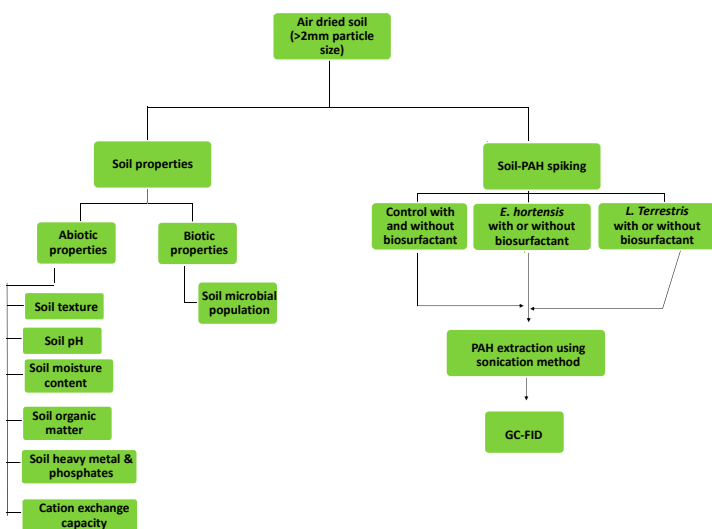


Figure 3 Flowchart of research methodology

RESULTS & DISCUSSION

Table 1: Soil properties

Soil sampleA	KETERING SOIL
pH	7.1 ± 0.02
Bacteria (cfu g ⁻¹)	3.6 × 10 ⁴
Fungi (cfu g ⁻¹)	5.6 × 10 ³
Actinomycetes (cfu g ⁻¹)	5.5 × 10 ⁴
Soil organic matter (%)	3.9 ± 0.3
Soil moisture content (%)	4.9±0.2
Fe (mg g ⁻¹)	15.56± 2.2
Mn (mg g ⁻¹)	0.27±0.004
Pb (mg g ⁻¹)	0.017±0.002
Zn (mg g ⁻¹)	0.025±0.002
P (mg g ⁻¹)	0.42±0.01

- Biosurfactant enhanced and accelerated removal of Fluoranthene and Phenanthrene
- E. hortensis* had higher tolerance limits and removed more PAH than *L. terrestris*
- Increase in microbial population indicates correlation between microbes and PAH removal by *E. hortensis* and *L. terrestris*

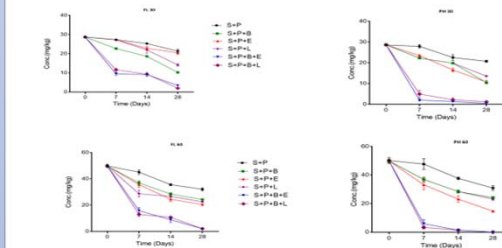


Fig 4: comparative removal of FL and PH from treatment after 4 weeks in both *Eisenia hortensis* and *Lumbricus terrestris*. All data are represented as means ± SD of triplicates. (where S=soil, P=pollutant, B= biosurfactant, E=*Eisenia*, and L=*Lumbricus*)

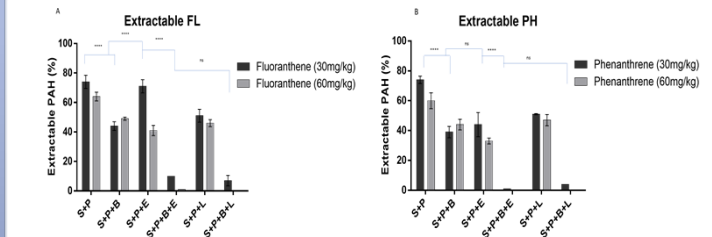


Fig 5 (A&B): A comparative overview of FL and PH removal between treatments in both *Eisenia hortensis* and *Lumbricus terrestris*. All data are represented as means ± SD of triplicates. **** represents a significant difference at P < 0.0001, * represents p < 0.05 and ns represents no significance.

CONCLUSION

Both epigeic and anecic species of earthworms have very good potentials to remove PAHs both 3 and 4 ringed hydrocarbons in the presence of rhamnolipid biosurfactant. Epigeic species appeared to be more tolerant to increased concentrations and environmental conditions compared to anecic species that were extremely sensitive to concentrations and environmental conditions around them. Their application to contaminated land does prove to be a promising and environmental friendly technique in removing hydrocarbons from soils. However a major limitation for using anecic earthworm species would be environmental conditions to which they are exposed, and also a large amount of both species would probably be needed on the field as well as sufficient substrate for their growth and general activity.

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